Pancreatic Cancer Cell DNA Content Correlates With Long-term Survival After Pancreatoduodenectomy

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The DNA content of 47 adenocarcinomas arising in the head of the pancreas from patients who had undergone successful pancreatoduodenectomy was measured. The DNA measurements of each tumor were made without knowledge of the clinical course by absorption cytometry performed on Feulgen-stained nuclei that had been disaggregated from pancreatic cancer tissue blocks. Forty-seven evaluable DNA distributions were obtained from specimens taken between 1975 and 1988. Of the 47 tumors, 19 (40%) were diploid and 28 (60%) were aneuploid cancers. The 19 patients with diploid cancers had a median survival time of 25 months. Median survival of the 28 patients with aneuploid cancers was 10.5 months. This difference was statistically significant ($p = 0.003$). A multivariate life table regression analysis demonstrated that the ploidy and proliferative index as determined by absorption cytometry were independent prognostic factors, as strong as or stronger than the number of positive nodes and tumor size. Thus cellular DNA content appears to be one of the most important predictors of survival in patients with adenocarcinoma of the head of the pancreas who have successfully undergone a pancreaticoduodenectomy.

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THERE IS CONTROVERSY as to what constitutes optimal therapy for patients with pancreatic cancer. Advocates of aggressive management point out that the operative mortality rate for pancreatoduodenectomy is falling. In addition there seems to be a small (2.5% to 20%) but consistent group of 5-year survivors among patients undergoing successful pancreatoduodenectomy for this disease.[1-7] The value of surgically exploring all patients who are thought to potentially have resectable pancreatic cancers has been questioned.[8-11] It is estimated that less than 1% of all of the patients with pancreatic cancer will actually survive for 5 years.[5,8-12] Even when newer preoperative staging modalities are used to screen for resectable cancers, palliative operations are still being performed on the majority of patients. Many of these patients who undergo surgical exploration and palliative bypass might be better palliated with less morbidity, deaths, hospital time, and expense by the use of percutaneously or endoscopically placed biliary stents.[13,14] If one could determine at the time of patient presentation which patients had a pancreatic tumor with biology favoring long-term survival, perhaps just these patients could be explored, leaving the larger group to be palliated nonoperatively.

The DNA contents of most pancreatic cancers can be measured before operation by absorption cytometry on cancer cells obtained by fine-needle aspiration.[15-21] Thus tumor DNA content could provide useful preoperative prognostic information for patients with pancreatic cancer. To evaluate this possibility, a retrospective study of the DNA content of 47 pancreatic cancers was carried out in patients who had undergone a pancreatoduodenectomy. By using standard life table methods, the effect of the measured DNA content on survival was determined.

Materials and Methods

Selection of Cases for Study

All patients who underwent pancreatoduodenectomy at this institution between 1975 and 1988 and from whom

Supported by the Department of Veterans Affairs.

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Accepted for publication September 24, 1990.

adequate tissue specimens were available were evaluated for inclusion in this study. During this period pancreatoduodenectomy was performed on all patients who were found at surgical exploration to be free of distant metastatic disease and whose tumors did not invade the adjacent major vascular structures. The microscopic slides from all cases were reviewed by two of us (RHH and JKB). Four criteria were established for inclusion of a case in this study. First there had to be evidence of origin in the head of the pancreas. Second the neoplasm had to have a malignant histology. Third the neoplasm had to demonstrate both epithelial and glandular differentiation. Fourth only patients who survived surgery were included. The first criterion could be fulfilled in one of two ways: (1) the neoplasm contained an *in situ* component in the pancreatic ducts, or (2) the bulk of the neoplasm was present within the head of the pancreas. Cases in which an *in situ* component was identified solely within the bile duct or duodenum were excluded. Occasional cases in which there was apparent *in situ* carcinoma in both the pancreatic and bile duct were included only if the tumor was clearly centered in the pancreatic parenchyma rather than around the bile duct. To fulfill the second criterion, that of a malignant histology, the neoplasm had to have stromal, perineural, or vascular/lymphatic invasion. To fulfill the third criterion, the neoplasms had to show evidence of epithelial differentiation and lumen formation by light microscopy. In cases in which the carcinoma was poorly differentiated, the glandular differentiation was confirmed with the mucicarmine stain. Fifty-one pancreatic cancers were identified that fulfilled these criteria. The cells from four of these neoplasms retained insufficient Feulgen stain for adequate DNA measurements, leaving evaluable DNA distributions from 47 of the pancreatic cancers. Two cases were mixed adenocarcinomas–neuroendocrine tumors, and one case was a mixed adenocarcinoma-small cell carcinoma. The remaining 44 neoplasms were pure adenocarcinomas.

Cell Preparation and DNA Measurements

A modification of the basic method of cell preparation of Hedley and co-workers was employed.[22,23] The following consecutive sections were cut from the cancer-containing tissue blocks: a 5-μm section, at least three 50- to 100-μm sections, and then a 5-μ section. The two 5-μ sections were stained with hematoxylin and eosin for confirmation of the presence of pancreatic cancer cells in the 50- to 100-μm sections. The 50- to 100-μm sections were then rehydrated in a sequence of two 15-minute washes in xylene, followed by washes in ethanol/xylene (50:50) to 100%, 95%, 70%, and 50% ethanol in water at room temperature. The sections were then incubated overnight in 0.25% Trypsin solution (Grand Island Biological Company, Grand Island, NY) containing 1.5 mm spermine tetrahydrochloride (Sigma, St. Louis, MO) at 37 C with shaking. After vortexing and filtration over a 60-μm mesh nylon screen, the single cells and nuclei obtained were placed onto slides along with chicken erythrocytes as an internal standard for DNA content. The cells were fixed in 1% paraformaldehyde and then stained by the Feulgen reaction. The amount of Feulgen stain bound to each nucleus is proportional to the DNA content of that nucleus. Acid hydrolysis for Feulgen staining was performed in 4 N NaCl at 28 C for 1 hour, followed by Feulgen-staining in Schiff's reagent (Sigma, St. Louis, MO) for 1 hour at room temperature.[24] Absorption cytometric DNA measurements were made with a Vickers M85 microdensitometer (Vickers Instruments, Malden, MA) with a 100×, 1.25 NA achromatic lens. A 0.4-μm spot was used for the absorbance measurements. Absorbance measurements were made at light wavelengths between 570 and 615 nm, with a spectral bandwidth of 15 nm and a 4% instrument glare level,[24] and the integrated absorbances were automatically corrected for optical errors due to stain darkness.[25]

The absorption-cytometric DNA measurements on each slide were made without knowledge of the survival status of the patient from whom the tumor was taken. For generation of DNA distributions, DNA measurements on at least 300 cells per tumor were performed in randomly selected microscope fields. After disaggregation, it was possible to morphologically distinguish many nontransformed lymphocytes, polymorphonuclear leukocytes, and fibroblasts. We did not measure the DNA contents of all of these cells, but selectively performed absorption cytometric measurements on intact nuclei showing epithelioid, or indeterminant, morphologic features. The DNA contents of some adjacent chicken erythrocytes, as well as nontransformed host lymphocytes and polymorphonuclear leukocytes, were measured in each microscopic field to establish internal standards for DNA content.[24,25]

The DNA histograms obtained by absorption cytometry separated into two groups. One group was observed to contain a single major peak that contained morphologically identifiable stromal and inflammatory cells as well as tumor cells, which had a diploid DNA content (2C) and was assigned a DNA index of 1.0. Aneuploid tumors, which formed the second group, consisted of specimens in which distinct peaks in addition to the diploid peak could be identified. The DNA index of the aneuploid tumors was calculated as the ratio of the DNA content of the abnormal DNA stemline to that of the diploid peak. Two of the cancers were classified as tetraploid (DNA index = 2 and having >10% S- and G2/M-phase tetraploid

cells). These tetraploid cancers were grouped with the aneuploid tumors. The DNA measurements also provide a gauge of the proportion of tumor cells that are in the different phases of the cell cycle (G0/G1, S, and G2/M phases), and these proportions were calculated from rectangular DNA distributions.[26] The proliferative index (PI) is the proportion of tumor cells in the S and G2/M phases of the cell cycle. Finally absorption cytometric DNA measurements can lead to the detection of rare tumor cells with DNA contents far above that of the G0/G1-, S-, and G2/M-phase cells of the "main" tumor stemline.[27] Because the presence of such cells might portend for a poor patient prognosis, the percentage of such ">G2/M cells" in each tumor also was measured.

Statistical Methods

The major statistical endpoint of this study was the duration of survival. Nonparametric estimates of the probability of survival were made by the product-limit method.[28] Differences between survival distributions were assessed for statistical significance by means of the log-rank statistic.[29] The prognostic effect of DNA measurements and of other dichotomous variables was expressed as a *hazard ratio*, in other words, the risk of death in those with the variable divided by the risk of death in those without. Thus hazard ratios greater than 1.0 imply an increased risk for those with the prognostic factor, and ratios less than 1.0 imply a decreased risk. For continuously distributed prognostic variables (*e.g.*, age), the hazard ratio was expressed per unit change (*e.g.*, change per year of increased age). Hazard ratios were estimated using the proportional hazards regression model.[30] To estimate hazard ratios while simultaneously controlling for other prognostic factors, we used the multivariate proportional hazards regression model.[30] The multivariate models focused on both the overall strongest independent predictors and the strongest independent preoperative predictors. To demonstrate multivariate analyses clinically, patients were classified into risk groups based on statistically significant prognostic factors. These risk groups have significantly different prognoses as a consequence of their arising from the multivariate analyses. All p values reported are two-sided.

Results

Patients

Of the 47 patients for whom we obtained interpretable histograms, 22 were men and 25 were women. The ages of these patients ranged from 33 to 74 years at the time of tumor resection. There were 37 white and 10 black patients. Current follow-up data were available for all patients (Table 1). All patients were discharged from the hospital after surgery except for one who died with liver metastases 2 months after resection. The liver metastases in this case were confirmed by postmortem examination. Thirty-four of the thirty-five patient deaths were directly attributable to recurrent cancer, with one patient dying of an apparent cerebrovascular accident 4 months after resection.

TABLE 1. *Age, Sex, Race, and Postresectional Survival Times (mo) of Pancreatic Cancer Patients Whose Tumor DNA Contents Were Measured*

Age Range at Resection	Race		Dead	Alive
	Black	White		
M				
50–59	2		3, 12	
60–69	2		2, 9	
30–39		2	10	24
40–49		1	9	
50–59		6	8, 11, 12, 13	15, 23
60–69		7	5, 8, 8, 32	21, 27, 127
70–79		2	12, 19	
F				
40–49	1		44	
50–59				
60–69	4		4, 6, 9, 23	
70–79	1			55
40–49		2	5, 11	
50–59		6	10, 12, 24, 26, 76	49
60–69		8	5, 12, 13, 23, 26, 69	16, 29
70–79		3	5	42, 44

Pancreatic Cancer DNA Contents, Pathology, and Patient Survival

The quality of the DNA histograms generated from the archival material was generally good, although the older material stained less intensely than did the more recent samples. This was demonstrated by an decreased absorbance ratio of the G0/G1 human diploid cells to chicken erythrocytes for nuclei harvested from the older tissue blocks. It also was indicated by the fact that evaluable DNA distributions were obtained from only three of seven tissue blocks from specimens obtained before 1980, whereas all of the 44 tissue blocks obtained in 1980 or later yielded evaluable distributions. There were 19 diploid and 28 aneuploid pancreatic cancers (Tables 2 and 3). None of the aneuploid cancers had DNA indices of less than 1. Twenty-six of twenty-eight aneuploid cancers had G0/G1 DNA peaks with cell numbers that made up 9% or more of their respective G0/G1 diploid DNA peaks (range, 9% to 91%, average of 39%). Two tumors were considered to be aneuploid on the basis of morphologically similar cells clustered at G0/G1 DNA peaks with indices of 1.69 and 1.74, even though these relatively rare aneuploid G0/G1 cells had cell numbers that were only 4%

TABLE 2. *Tumor Size, Cell Cycle Parameters, and Survival Status of Patients With Diploid Cancers After Successful Pancreatoduodenectomy*

DNA Index	Survival Time (mo)	Survival Status	Size (cm)	Nodes	% G1	% S	% G2/M	% > G2/M
1.00	5	Dead	3	2/7	84	7	9	0.3
1.00	8	Dead	3	1/25	86	8	4	2
1.00	11	Dead	1.5	11/25	88	7	5	0.3
1.00	12	Dead	3.5	1/7	95	2	3	0
1.00	12	Dead	3.5	3/12	92	4	4	0
1.00	19	Dead	2	2/5	97	0	3	0
1.00	23	Alive	2	0/10	87	10	2	1
1.00	23	Dead	4	4/8	90	4	6	0
1.00	24	Alive	4	2/10	94	1	5	0
1.00	25	Dead	5	2/14	80	4	15	1
1.00	26	Dead	7	0/5	97	2	1	0.3
1.00	29	Alive	2.5	0/6	88	2	10	0.3
1.00	44	Alive	2	0/0	91	1	9	0
1.00	44	Dead	1.5	0/27	90	5	5	0
1.00	49	Alive	3	1/12	82	9	7	1
1.00	55	Alive	2	0/8	94	2	4	0
1.00	69	Dead	1.5	2/14	95	3	2	0
1.00	76	Dead	2	0/21	94	5	1	0
1.00	127	Alive	4	0/16	90	4	5	1

and 6%, respectively, of their diploid G0/G1 DNA peaks. Strong support for the aneuploid classification of these tumor was provided by the finding in both cases of morphologically identical cells with tightly clustered DNA contents at the expected G2 DNA values for these tumors, as well as the presence of cells with DNA values within the appropriate ranges for their S-phase compartments.

The PI of the diploid cancers ranged from 3% to 16% (Table 2), whereas the PIs of the aneuploid cancers ranged from 5% to 38% (Table 3). Nine of the nineteen diploid

TABLE 3. *Tumor Size, Cell Cycle Parameters, and Survival of Patients With Aneuploid Tumors After Successful Pancreatoduodenectomy*

DNA Index	Survival Time (mo)	Survival Status	Size (cm)	Nodes	% GI	% S	% G2/M	% > G2/M
1.57	2	Dead	3	4/6	72	14	10	4
1.82	3	Dead	4.5	2/13	92	7	1	0
1.76	4	Dead	4	1/7	83	16	0	1
1.89	5	Dead	4	0/5	72	8	15	5
1.81	5	Dead	3	7/11	70	17	13	0
2.25	5	Dead	2.5	3/10	85	7	7	1
1.81	6	Dead	4.5	6/13	65	20	14	2
1.73	8	Dead	6.5	2/19	61	32	4	3
1.45	8	Dead	5.5	6/60	78	21	1	0
1.56	9	Dead	3	0/0	60	19	19	1
2.06*	9	Dead	4	2/5	86	9	2	3
1.75	9	Dead	2	5/17	63	13	25	0
1.69	10	Dead	5	1/6	72	16	12	0
1.88	10	Dead	3.5	3/6	89	8	3	0
1.87	11	Dead	6	2/4	78	14	7	2
1.92	12	Dead	3	0/8	80	10	10	0
1.74	12	Dead	2.5	1/8	83	13	2	2
1.56	12	Dead	2.5	2/14	69	19	8	4
1.67	13	Dead	1.5	0/3	76	9	12	2
1.90	13	Dead	3	1/12	86	12	1	1
1.61	15	Alive	0.5	0/0	89	0	11	0.5
1.98*	16	Alive	2	0/7	83	3	13	0
1.67	21	Alive	2	0/10	81	12	6	1
1.79	23	Dead	2	2/3	91	5	4	0.7
1.85	24	Dead	3.5	9/19	82	11	7	0
1.84	27	Alive	1.5	2/11	90	9	1	0
1.66	32	Dead	1	0/13	77	16	6	1
1.79	42	Alive	2.5	3/8	95	3	2	0

* Tetraploid cancers.

tumors had a small percentage of cells (0.3% to 2%, Table 2) with DNA contents higher than the main stemline (>G_2M), whereas 17 of the 28 aneuploid cancers had such >G_2/M cells (0.5% to 5%, Table 3).

Figure 1A and B shows photomicrographs of two different pancreatic cancers. Both tumors show invasive adenocarcinomas with marked nuclear hyperchromasia. Although both appear relatively similar histologically, Figure 2A and B illustrates that the DNA distributions of these pancreatic cancers are in fact quite different. The tumor in Figures 1A and 2A had a diploid DNA content with a G0/G1 peak having a DNA index of 1, and a corresponding G2/M peak with a DNA index of 2. This tumor had a relatively high PI of 16% (Fig. 2A). The tumor in Figures 1B and 2B was aneuploid, with a DNA index of 1.56 and a PI of 27%. The high PI of the diploid tumor may be the explanation for the hyperchromatic staining of many of the cell nuclei in this tumor, which resembled the intense nuclear staining of the aneuploid cancer. The DNA measurements allowed separation of two tumors that could not be separated histologically into different classes. It is of interest that the patient with the aneuploid cancer (Figs. 1B, 2B) succumbed to progressive disease 12 months after resection, whereas the patient with the diploid cancer (Figs. 1A, 2A) has remained tumor free for more than 4 years after resection.

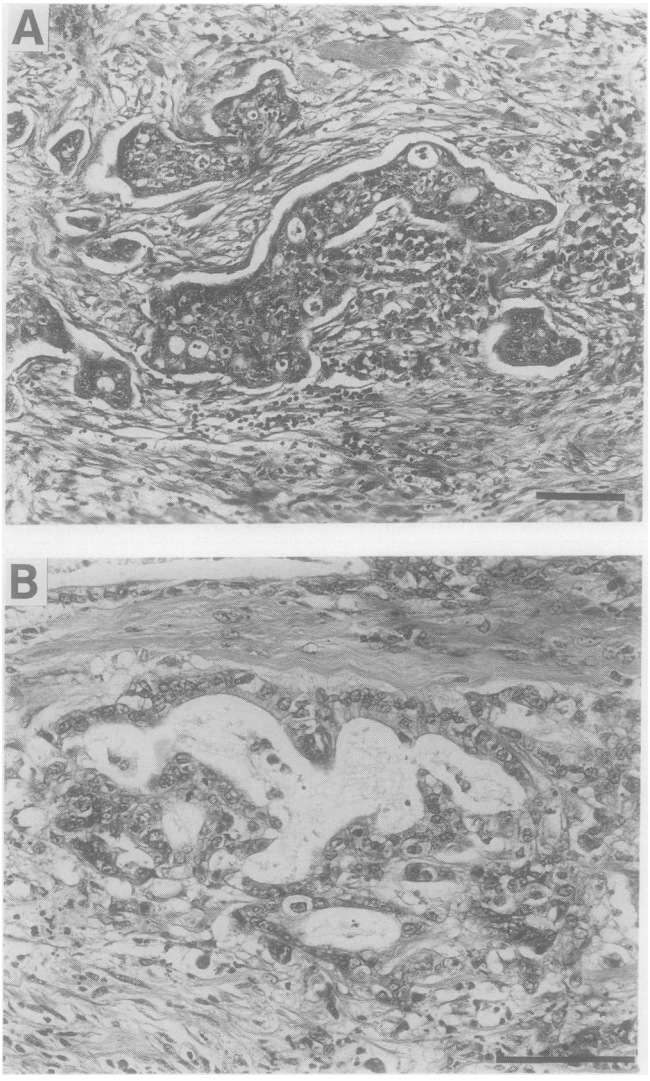

FIG. 1A and B. Hematoxylin and eosin–stained 5-μm sections of pancreatic adenocarcinomas in two pancreatoduodenectomy specimens. A, Section of a 3-cm tumor resected from a 59-year-old white woman shows deep invasion, marked nuclear atypia, and hyperchromasia. B, Section of a 2.5-cm tumor resected from a 50-year-old white man also shows deep invasion, marked nuclear atypia, and hyperchromasia. *Bar*, 100 μm.

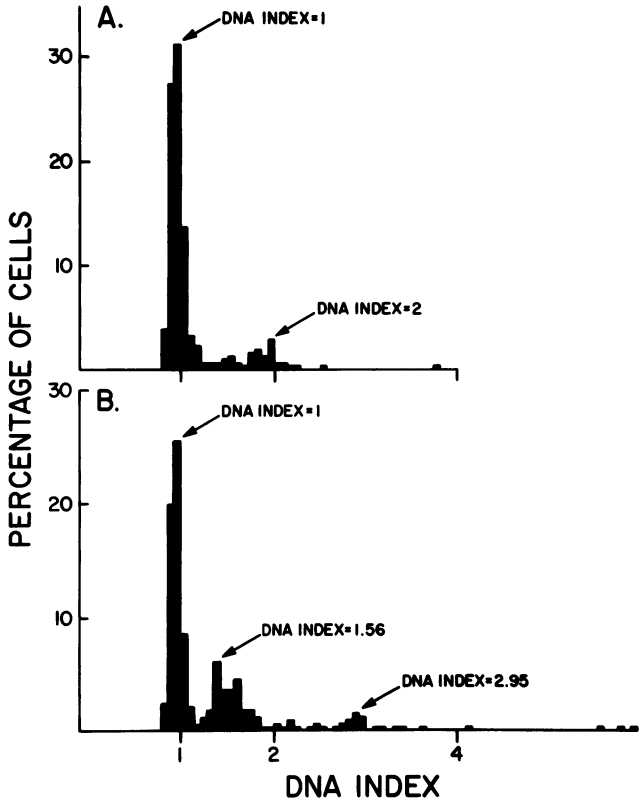

FIG. 2A and B. DNA distributions of the two pancreatic tumors shown in Fig. 1. A, DNA distribution of the tumor shown in Fig. 1A. This tumor was diploid (DNA index, 1), with a PI of 16%. B, DNA distribution of the tumor shown in Fig. 1B. This tumor contains both diploid cells (DNA index, 1) and an aneuploid tumor stemline (DNA index, 1.56), with a PI of 27%. This patient died of progressive pancreatic cancer 12 months after resection, whereas the patient with the diploid cancer remains tumor-free 4 years after resection.

Univariate analysis demonstrated that the hazard of death for patients with aneuploid tumors was 3.3 times greater than that for patients with diploid tumors (Table 4). This hazard ratio is significantly greater than 1 ($p = 0.003$). For the patients with diploid pancreatic cancers, the median survival time was 25 months, whereas patients with aneuploid tumors had a median survival of only 10.5 months. The percentage of S-phase cell, the percentage

of cells with >G_2M DNA content, a high PI, and the presence of lymph node metastases all had unfavorable prognostic influences in univariate analysis when patients with both diploid and aneuploid tumors were considered together or separately (Table 4). As expected, the univariate analysis also showed that age, race, sex, and year of resection were *not* prognostic for long-term survival (Table 4).

Several multivariate models were used for prediction of survival time (Table 5). Aneuploid *versus* diploid DNA content remained highly prognostic even after adjustment for tumor size, PI, and nodal involvement (Table 5). The risk of death for patients with aneuploid tumors appeared to be approximately four times that for patients with diploid tumors, even after adjustment for the size of the tumor. After ploidy status and tumor size were accounted for, the presence of cells with DNA contents >G2/M, age, sex, and race did not appear to be statistically significant prognostic variables. Patients diagnosed and treated more recently did not have a significantly more favorable prognosis than did earlier patients (Table 5). The strongest preoperative prognostic factors were ploidy status, tumor size, and PI.

The estimated 3-year survival rates for patients with diploid and aneuploid cancers were 51% and 8%, respectively. The difference in survival is shown graphically in Figure 3. This difference is statistically significant ($p < 0.003$, Table 4). Placement of the two patients with the tetraploid cancers in the diploid rather than the aneuploid group did not appreciably alter the results of the statistical analysis. Also removal of the one patient who apparently did not die of recurrent pancreatic cancer from consideration did not substantially change the results of the statistical analysis.

TABLE 4. *Estimated Hazard Ratios for Death, With Significance Levels, When Prognostic Factors are Considered Individually*

Variable	Hazard Ratio	95% Confidence Limits	p*
Aneuploid *vs.* diploid	3.28	1.48–7.24	0.003
% G0/G1	0.93	0.89–0.96	<0.001
% S	1.11	1.05–1.16	<0.001
% G2/M	1.05	0.98–1.13	0.14
% > G2/M	1.46	1.12–1.89	0.004
Σ (S + G2/M + >G2/M)†	1.08	1.04–1.12	<0.001
PI (S + G2/M)†	1.08	1.04–1.12	<0.001
Tumor size/cm‡	1.28	1.04–1.56	0.02
>2.5 cm *vs.* ≤ 2.5 cm	2.75	1.36–5.60	0.005
Positive nodes/node§	1.15	1.02–1.28	0.02
Nodes, positive vs. negative	3.26	1.42–7.62	0.006
Age/year	0.99	0.95–1.02	0.49
White *vs.* nonwhite	0.50	0.23–1.08	0.08
F *vs.* M	0.86	0.44–1.71	0.67
Year of diagnosis	1.02	0.92–1.13	0.69

* p value for hypothesis that the hazard ratio equals 1.0.
† Hazard ratio for each 1% of PI or Σ(S + G2/M + >G2/M).
‡ Hazard ratio for each centimeter of tumor diameter.
§ Hazard ratio for each positive lymph node.
PI, proliferative index.

TABLE 5. *Estimated Hazard Ratios for Death, With Significance Levels, for Multivariate Proportional Hazards Regression Models**

Term	Hazard Ratio	95% Confidence Limits	p†
Aneuploid	2.85	1.10–7.34	0.03
PI	1.05	1.00–1.10	0.07
Tumor > 2.5 cm	2.98	1.40–6.33	0.01
Aneuploid	4.88	1.96–12.16	<0.001
Year of diagnosis	0.95	0.84–1.08	0.44
Tumor size/cm	1.38	1.11–1.70	0.004
Nodes, positive *vs.* negative	2.44	1.01–5.87	0.05
Aneuploid	2.48	1.00–6.27	0.05
Tumor > 2.5 cm	2.47	1.14–5.35	0.02
PI	1.05	1.00–1.10	0.03
Aneuploid	4.18	1.83–9.54	<0.001
Tumor > 2.5 cm	3.47	1.66–7.24	<0.001

* Each panel represents a separate multivariate model.
† p value for hypothesis that the hazard ratio equals 1.0.
PI, proliferative index.

Relationships Between the DNA Measurements, Tumor Size, and Patient Survival

Table 5 shows that tumor ploidy levels and size were statistically significant and independent prognostic variables for the length of survival after successful resection for pancreatic cancer. Based on the multivariate analysis in Table 5 (last panel), risk groups can be identified using ploidy and tumor size. Figure 4 is a Kaplan-Meier plot of the survival times of the risk groups with small and large diploid and aneuploid cancers. It can be seen that the patients with small (≤2.5 cm) diploid cancers have 2-

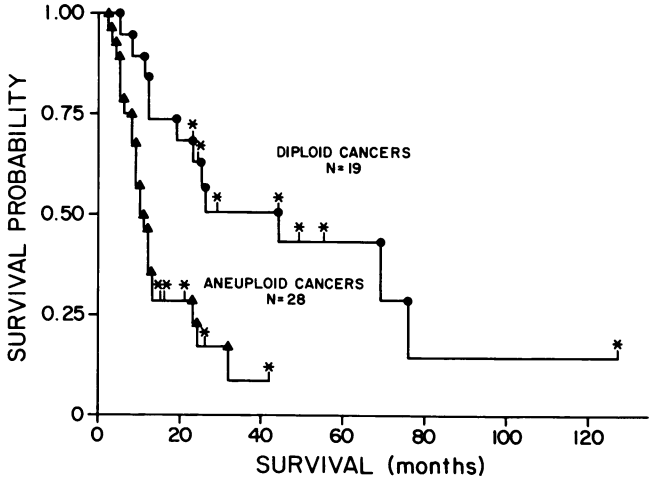

FIG. 3. Kaplan-Meier survival curves for the 19 patients with diploid (●) and the 28 patients with aneuploid pancreatic carcinoma (▲) who survived pancreatoduodenectomy. Time of last follow-up of surviving patients (*).

year survivals of 78%. The patients with small (≤2.5 cm) aneuploid and large (>2.5 cm) diploid cancers had intermediate 2-year survivals of 44% and 50%, respectively. Statistical tests of these differences are shown in Table 5, last panel. Only 1 of the 16 patients with aneuploid pancreatic cancers greater than 2.5 cm survived longer than 1 year, and this patient succumbed to recurrent disease 24 months after resection (Fig. 4). None of the nine patients with aneuploid tumors 4 cm or larger survived for longer than 11 months after resection (Table 3). Because the survival times plotted do not include the time of hospitalization for surgery (average of 1 month) and subsequent admissions for the complications of progressive disease and terminal care, it would seem that the patients with large aneuploid pancreatic cancers have very limited lifespans outside of the hospital after resection.

Discussion

We undertook the present work in an effort to determine whether cellular DNA content, as measured by absorption cytometry, is a valid prognostic indicator for carcinoma of the pancreas. This technique of DNA measurement, rather than flow cytometry, was selected because it can readily be applied to pancreatic cancer cells obtained preoperatively by fine needle aspiration.[21] Also absorption-cytometric DNA measurements seem to pose fewer problems with the artifactual interpretation of cellular debris as S-phase cells and the loss of rare cell populations from the DNA distribution.[27,31]

Measurement of DNA content, performed either by absorption or flow cytometry, allows determination of whether a tumor is diploid or aneuploid and also indicates the proportion of proliferating tumor cells (expressed as PI). On theoretical grounds, one would suppose that patients with aneuploid tumors, or tumors with a high PI, should have a worse prognosis; however, clinical experience with measurements of DNA content for a wide variety of human cancers has shown that this is not always the case. Many clinically benign tumors have an aneuploid DNA content.[32-36] For carcinomas of the thyroid and gallbladder, carcinoid tumors, and node-positive breast cancers, ploidy levels or the PI give little independent prognostic information.[37-40] For colorectal cancers,[41-44] melanoma,[45] and node-negative breast cancers,[46] however, the DNA measurements provide important and useful prognostic information. Surprisingly for some cancers, such as neuroblastomas, aneuploidy in a tumor is a favorable prognostic sign.[47] Thus the value of DNA content as a prognostic indicator must be assayed independently for each type of human cancer.

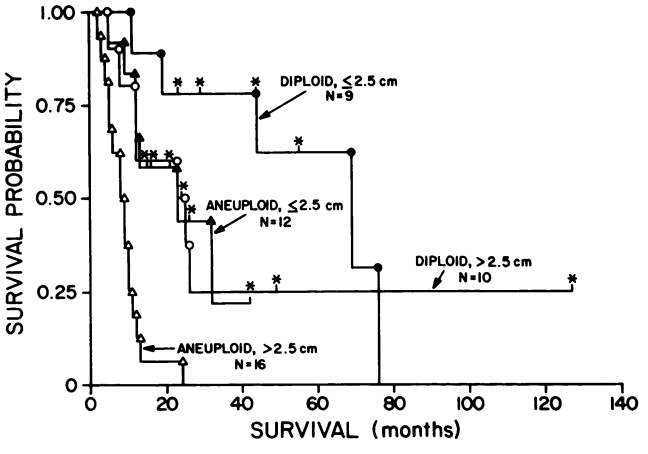

FIG. 4. Kaplan-Meier survival curves for the 9 patients with small (≤ 2.5-cm) diploid pancreatic tumors (●); the 12 patients with small aneuploid carcinomas (▲); the 10 patients with large (> 2.5-cm) diploid cancers (○); and the 16 patients with large aneuploid cancers (△). Time of last follow-up of surviving patients (*).

In the present study, tumor aneuploidy correlated with poor survival (Tables 4, 5). Only 8% of the patients with aneuploid cancers survived for 3 years, whereas 51% of the patients with diploid cancers were alive at 3 years (Fig. 3). Multivariate analyses demonstrated that ploidy levels (aneuploid *vs.* diploid DNA status), the PI, nodal involvement, and the size of the carcinoma were statistically significant prognostic indicators (Table 5). The age, sex, and race of the patients were not independent prognostic indicators of survival after correction for tumor ploidy level, the PI, nodal involvement, and tumor size.

Joensuu et al.[48] found in a flow cytometric study that only 3 of 15 resected pancreatic cancers had aneuploid DNA content, whereas 35 of 47 nonresected pancreatic cancers were aneuploid. Although none of the patients in this series survived longer than 2 years, the patients with diploid pancreatic cancers lived longer than patients with aneuploid cancers. This finding led these authors to conclude that the modest survival advantage observed for the group of patients undergoing surgical resection was due not to an actual benefit of surgery, but to the fact that the tumors with more intrinsically favorable biologic behavior were being selected for resection.[48]

In a recent flow cytometric and image analysis DNA (Feulgen) study, Weger et al.[49] found that 76 of 77 pancreatic cancers (50 of which were resected) had "nondiploid" DNA contents. Furthermore these authors concluded that patients with "triploid" DNA contents had an especially poor prognosis. We believe the high rate of aneuploid pancreatic cancers found by Weger et al., when compared with the rates of aneuploidy found in our study (Tables 3, 4) and that of Joensuu et al.,[48] may be due to technical artefacts. Selection of the relatively rare cells in diploid tumors with >G2/M DNA contents (Table 2) for image analysis DNA measurements could give an erroneous impression of aneuploidy. Also Weger et al.[49] analyzed material from tissue blocks obtained between 1972

and 1988. We found that Feulgen DNA measurements obtained from tissue blocks 8 years or older were often unreliable and showed widening of the 2C and 4C DNA peaks. Optical errors such as glare can cause dispersion of DNA measurements.[24,25] Finally flow cytometric studies of the DNA contents of disaggregated pancreatic cells can lead to spurious, "false aneuploid" peaks around the 2C and 4C DNA values secondary to autolysis of nuclei by pancreatic enzymes.[50] Thus it is possible that only the "triploid" cancers of Weger et al. are truly aneuploid. If this is the case, the relatively poor prognosis observed for this subset of patients[49] would be consistent with the poor survival results for patients with aneuploid pancreatic cancers found in our study (Fig. 3) and by Joensuu et al.[48]

The finding that patients with diploid cancers survived longer than patients with aneuploid cancers supports the hypothesis that the biologic behavior of pancreatic cancer plays a large part in the duration of patient survival.[48,49] We believe that this finding does not imply, as Joensuu et al. suggest, that resectional surgical therapy should not play an important role in treatment for the majority of pancreatic cancers for which the operation is possible.[48] Admittedly the two long-term deaths from recurrent pancreatic cancer, at 69 and 76 months after resection, leave open the possibility that at least some of the apparent benefits of surgery with regard to survival are due to lead time bias. Nevertheless there are five other patients in this series who are currently approaching or are alive more than 4 years after resection. We hope that at least some of these patients will be "cured," and there is one 10-year tumor-free survivor. Regardless of the eventual outcomes of these patients, however, one can only speculate what their clinical courses would have been if their cancers had not been resected.

A subset of patients with "large" (>2.5 cm) aneuploid cancers were identified who had uniformly poor survivals even after "curative" resections (Table 3, Fig. 4). Because DNA measurements by absorption cytometry can be performed on pancreatic cancer cells obtained by fine-needle aspiration,[24] the combination of these DNA measurements with preoperative imaging estimates of tumor size may have future potential for the selection of therapy for certain pancreatic cancer patients. Specifically the DNA and tumor size measurements may eventually allow the identification of patients with pancreatic cancers that are "resectable" by currently employed criteria, but who are not curable and for whom pancreatoduodenectomy provides little or no real benefit (Table 3, Fig. 4). Also these measurements may allow the identification of other pancreatic cancer patients with tumors currently judged "unresectable," for whom more extensive attempts at regional surgical resection, including resecting portions of the portal and superior mesenteric veins, could be justified. Finally this information may allow more accurate stratification of pancreatic cancer patients in controlled studies to determine which of these patients may benefit from adjuvant therapy.[51,52]

The clinical application of preoperative imaging techniques and DNA measurements as a guide for selecting patients for surgical resection at this time would be premature. The results of this retrospective study in which we work with fixed material should be confirmed and extended prospectively with a larger number of patients before the DNA measurements can be used with confidence as a basis for clinical decisions.

Acknowledgment

The help and support of the Tumor Registry of the Johns Hopkins Oncology Center is gratefully acknowledged.

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